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## Abstract

The studies reported here have examined neutrophil activity and function in 1) human subjects engaged in a week-long Bushmaster exercise, a field operation in which military medical students are involved in a simulation of MASH operations and 2) rats subjected to cold stress over a four hour exposure period.

Military medical students engaged in four Bushmaster exercises have been studied twice before and once immediately following the exercise. A final measurement was made approximately two weeks following exercise. The findings representing upto 19 subjects suggest that the exercise was associated with a time related increase in neutrophil respiratory burst capacity as measured in nitroblue tetrazolium reactions of chemoattractant stimulated neutrophils. Measures of neutrophil adherence were variable compared to matched control subjects and failed to reflect an exercise effect. Similarly, neutrophil migration in both random and chemotaxis, and total white blood cell and neutrophil numbers in the circulation were unchanged compared to control. The findings of increased oxidative burst reaction following stimulation with FMLP is suggestive of a priming or activation effect of these cells by activities related to the exercise..

In a companion study, rats were subjected to four hours of exposure to 4°C and physical and neutrophil function parameters were measured at the conclusion of the exposure period for comparison to response parameters of animals maintained at 25°C temperatures. The data indicated that the body temperature, total white blood cell numbers and numbers of neutrophils were reduced following cold exposure compared to matched controls. The functional parameters of oxidative burst and migration of neutrophils from cold exposed rats were depressed compared to matched control rats. In contrast, the measures of neutrophil adherence and degranulation of primary granules as measured by the concentration of vitamin B12 binding protein in rat serum were unchanged by cold exposure compared to controls. Taken together, these data suggest that cold exposure in the rat may alter the ability of neutrophils to respond functionally to certain inflammatory challenges and additional studies will be required to define the risks of cold exposure in the rat model.

## I. Introduction

The effects on immune system status and function have been of interest for many years, but it is recently that this topic has received extensive systematic research attention. Studies of acute and chronic stress with animal and human subjects have suggested that stress can affect immune system functioning by reducing cells' ability to proliferate when stimulated by a mitogen, by increasing the likelihood of reactivation of latent viruses, and, in some cases, by affecting the numbers of leukocytes and various subpopulations of lymphocytes (1). Though as a whole, unsystematized behavioral immunology has emerged as a rapidly developing field of scientific inquiry directed, in part, at better understanding the relationships between psychological and physiological events associated with stress on the one hand and immune function and health on the other.

During the past decade, research has clearly established links among the nervous, endocrine, and immune systems as well as demonstrating psychological influences on immune function and status. Research has indicated that immune responses can be conditioned (2,3) and several studies have shown that stress affects the strength of immune response to a variety of stimuli (1). Studies have chiefly used lymphocyte proliferation measures in this work, counting mitogen - induced replicates of T or B cells and relating proliferative ability to stress or other conditions. Other measures have been used, including counts of lymphocyte subpopulations, natural killer cell activity ( lysis of target cells ), quantitation of antibody titers to latent viruses, tests of delayed hypersensitivity, and tumor rejection. Choice of measures in these studies is based on several factors, and has not led to uniform study of all aspects of immune function; instead, some aspects of immune response have been more fully studied than others. A relatively neglected area is the effect of stress on neutrophil, or polymorphonuclear granulocyte ( PMN ), activity and function.

One study examined the effects of a stressful 77 - hour sleepless vigil on phagocytic capacity of neutrophils. Rate of phagocytosis was lower during the vigil than before or after it, but some evidence of adaptation by the end of the vigil was also reported. Plasma cortisol and urinary catecholamines appeared to be negatively correlated with phagocytosis. A second study (4) measured PMN adherence before and immediately after a 48 - hour sleepless period and five days afterward. They found no evidence of significant changes in adherence. However, measures in this study were taken before and after the vigil: no intermediate measures during the sleepless period

were reported. It is therefore possible that observations of meaningful changes were obscured by adaptation towards the end of the session.

Neither of these studies can be considered conclusive regarding the effects of stress deprivation on neutrophil activity. Sample sizes were small, little information about the subjects was reported, and the difficulty in separating the effects of stressful tasks and of sleep deprivation cloud interpretation of the findings.

As the field evolves it is necessary to examine aspects of immunity not commonly studied and stressors that more accurately reflect stressors that might be experienced in real - world settings (5). Our approach has been to study the neutrophil, a basic, non - specific form of immune defense against bacterial incursion, and to examine the effects on this cell in two biological systems of stress effects including 1) a week - long Bushmaster exercise, a field operation in which military medical students are involved in a simulation of MASH operations and 2) a rat model of cold exposure.

The Bushmaster exercise combines several forms of stress common in actual operations, including sleep deprivation, physical exercise, threat, uncomfortable living conditions, and evaluation apprehension. Clearly, it will not be possible to separate out the effects of these various stressor conditions so that we can determine those that have effects on neutrophil function. However, the naturalistic aspects of the exercise and the control afforded both in terms of conditions such as nutrition and of experiences during the exercise provide the opportunity of studying a real - world event under relatively well - controlled conditions. The Bushmaster exercise is held three times each year; during this reporting period, we studied two of these sessions making a total of four sessions studied from inception to date.

The rat model of cold stress has been incorporated in these studies in an effort to correlate findings with human subjects and to investigate selected parameters of neutrophil function following an experimental manipulation to induce an inflammatory challenge which would not be possible to study under controlled conditions in human subjects.

## II. Progress Report

### A. Research Objectives:

The primary goal of our research is to investigate the effects of 1) psychological and physical stress in human subjects during and after a military medical field exercise; and cold stress in a rat model, on neutrophil functions, including cells' ability to migrate, adhere, and undergo respiratory burst. The relationships among adrenal hormones, stress, and neutrophil function will also be investigated. The Bushmaster exercise, is required of all fourth year medical students at USUHS and is held three times each year. Because of limits on our ability to assay large numbers of samples of fresh blood, and because each exercise session may differ slightly, our strategy is to assess stress and neutrophil activity from several Bushmaster sessions and combine the data across a two - year period. The effect of cold stress on rats will be evaluated after four hours exposure to 4°C and compared to responses of rats maintained at 25°C. Because of the limited number of cells available from each rat, studies were conducted using neutrophils collected from blood pooled from 3 - 4 rats.

### B. Procedures:

Since the inception of this project, we have collected data from five Bushmaster exercise sessions held near San Antonio, Texas. Our first assessment was a pilot study, designed to test the feasibility of procedures adopted due to logistic difficulties in collecting data. A total of five medical students and four control subjects participated, and we were able to collect pre - exercise, and post - exercise samples ( upon returning to campus ) on five of the medical students subjects. Controls provided samples once, at the time of the pre - exercise assessment for Bushmaster participants. The pilot was successful in establishing logistical arrangements and procedures for subsequent sessions, and findings from these subjects were of some interest.

Following the initial pilot study, we have successfully completed study of 19 additional subjects participating in four Bushmaster sessions. Blood samples were evaluated for total white blood cell and neutrophil numbers. Subsequently, neutrophils were separated from blood by density gradient methods and the purified cells were evaluated for adherence, migration and oxidative burst reaction.

Studies were also conducted using specific pathogen free rats weighing 220-264 grams. Rats were subjected to 4°C temperature for 4 hours after which peripheral blood was collected from the abdominal aorta of three to four anesthetized animals (ketamine hydrochloride at 40 mg/kg) and pooled. Peripheral blood from three to four matched control rats maintained under identical conditions at 25°C was also collected for neutrophil studies.

Studies of neutrophil function were conducted using cells isolated from the peripheral blood by a combination of density gradient and dextran sulfate techniques (6). These isolated neutrophils were used for studies of adherence, migration and oxidative burst reactions. In addition to these studies, blood serum was collected from rats held at 4°C or 25°C for evaluation of vitamin B12 binding protein.

Adherence to glass surfaces was accomplished by placing 200 µl of media containing  $2 \times 10^6$ /ml neutrophils in one well of a 12 well glass ring slide. The slides were incubated for 30 minutes at 37°C in a humidified CO<sub>2</sub> - air atmosphere. The wells containing cells were gently rinsed and the adherent cells were fixed in methanol and stained for microscopic evaluation of the number of neutrophils adhering to the glass surface. Adherence of rat neutrophils followed that described for human cells.

Neutrophils were evaluated for random migration and chemotaxis using N - formyl - methionyl - leucyl - phenylalanine (FMLP) as a stimulant at  $1 \times 10^{-7}$  M in a 48 well modified Boyden chamber. The chamber was set up with the lower portion of the chamber containing either FMLP ( chemotaxis ) or culture media ( random migration ). A filter membrane with 5.0 µm size pores was placed over the lower portion of the chamber and neutrophils at  $2 \times 10^6$  cells/ml were placed in the upper wells of the chamber. Cells were allowed to migrate for one hour at 37° C after which the filter membrane was removed and stained before evaluating for numbers of cells migrating toward the chemoattractant using an Optimax image analyzer. The procedure for migration of rat neutrophils was the same as for human cells except that a 3.0 µm size filter membrane was used and the cells were allowed to migrate for 1.5 hours.

Deficiencies of oxidative metabolism are screened with the nitroblue tetrazolium dye (NBT) test, which is based on the ability of products of oxidative metabolism to reduce yellow, soluble NBT to blue-black formazan, an insoluble material that precipitates intracellularly and can be seen microscopically. Briefly, 200 µl of media

containing  $2 \times 10^6$  neutrophils/ml were incubated with media or FMLP ( $10^{-7}M$ ) and 200  $\mu$ l NBT for 10 minutes. The cells were then placed in cytobuckets and centrifuged for 5 minutes at 500 X G. Following centrifugation, the cells were fixed, stained and evaluated for number of cells undergoing an oxidative burst reaction. The procedure for evaluating oxidative burst reactions in rat neutrophils was the same as for human cells. Burst reaction for both human and rat neutrophils were scored on the intensity of the reaction using a 0 to 4+ scale.

### C. Results and Discussion:

#### 1. Bushmaster Exercise Subjects

Results of the hematologic and neutrophil studies of 19 Bushmaster subjects representing four exercise sessions are presented in Tables 1, 2 and 3. Study subjects were evaluated at time points before and after the Bushmaster exercise to include once at least two weeks before commencement of the exercise ( Pre 1 ), once at 2 - 5 days prior to the exercise ( Pre 2 ), once upon return to the Washington, DC area from San Antonio, Texas ( Post 1 ) and once approximately two weeks following the exercise ( Post 2 ). Although it would have been desirable to test these subjects during the exercise while engaged in their actual day-to-day activities, logistical limitations precluded performance of neutrophil studies. Nevertheless, blood serum was collected during this time to evaluate for levels of stress hormones. Evaluation of psychological stress profile of the study subjects and serum levels of cortisol and catecholamines were performed as part of a companion contract held by the Uniformed Services University of the Health Sciences ( Contract number N00014 - 88 - AF - 00001 ).

Data in Table 1 presents results (mean  $\pm$  SEM) of the hematologic response of Bushmaster subjects at each of the test periods evaluated before and after the exercise sessions. Results indicate that total white blood cell numbers and total numbers of circulating neutrophils were not different when compared to any of the test periods evaluated or to the cell numbers for matched control subjects evaluated at the same time. All hematologic values were within normal ranges.



**TABLE 1.** White Blood Cell (WBC) and Neutrophil Numbers From Bushmaster and Control Subjects at Pre- and Post Exercise Periods

Study Subjects	<u>PRE-EXERCISE 1</u>	<u>PRE EXERCISE 2</u>	<u>POST EXERCISE 1</u>	<u>POST EXERCISE 2</u>
	<u>TOTAL WBC Numbers (x 10<sup>3</sup>/μl)</u>		<u>Mean ± SEM (No. of Subjects)</u>	
Bushmaster	9.22 ± 0.53 (19)	8.59 ± 0.57 (18)	8.15 ± 0.27 (15)	8.96 ± 0.79 (7)
Control	9.20 ± 0.84 (9)	9.03 ± 0.95 (8)	8.76 ± 0.85 (6)	ND
	<u>Neutrophils Numbers (x 10<sup>3</sup>/μl)</u>		<u>Mean ± SEM (No. of Subjects)</u>	
Bushmaster	3.28 ± 0.35 (19)	3.37 ± 0.33 (18)	3.13 ± 0.37 (15)	3.35 ± 0.42 (4)
Control	3.38 ± 0.44 (9)	4.05 ± 0.72 (8)	3.19 ± 0.71 (6)	ND

The ability of Bushmaster neutrophils to migrate either randomly or toward a chemoattractant (Table 2) was found to be no different than the response of neutrophils from the matched controls indicating that migration capability may not represent a function altered by the psychological and physical stress induced by the Bushmaster exercise.

**TABLE 2.** Random and Chemotactic Migration of Neutrophils From Bushmaster and Control Subjects at Pre- and Post Exercise Periods

	<u>Migration Response</u>			
Study Subjects	<u>PRE-EXERCISE 1</u>	<u>PRE EXERCISE 2</u>	<u>POST EXERCISE 1</u>	<u>POST EXERCISE 2</u>
	<u>Random Migration</u>		<u>Mean ± SEM (No. of Subjects)</u>	
Bushmaster	34.2 ± 4.9 (15)	33.1 ± 6.3 (14)	18.0 ± 2.0 (14)	19.2 ± 1.9 (7)
Control	30.2 ± 7.1 (6)	33.6 ± 6.1 (8)	17.8 ± 4.9 (4)	ND
Percent Change	+11.7%	-1.5%	+1.1%	---
	<u>Chemotaxis (FMLP -10<sup>-7</sup> M)</u>		<u>Mean ± SEM (No. of Subjects)</u>	
Bushmaster	122.1 ± 15.3 (15)	151.4 ± 9.0 (14)	137.7 ± 7.0 (14)	124.8 ± 6.8 (7)
Control	122.4 ± 18.7 (6)	148.5 ± 13.8 (8)	138.8 ± 23.1 (4)	ND
Percent Change	-0.2%	+1.3%	-0.8%	---

Table 3 presents results of neutrophil adherence and oxidative burst reactions of Bushmaster subjects at time periods before and after exercise. Although, the adherence characteristics of cells from Bushmaster subjects tended to increase with time approaching and immediately after the exercise, the variability in responses between test days and between test subjects prevents any interpretation of exercise effects on adherence characteristics. The observed variability also negates a previous observation that the ability to adhere, seen with neutrophils from a small group of Bushmaster subjects (four subjects) evaluated early in the study, was altered after exercise.

**TABLE 3** Adherence and Oxidative Burst Response of Neutrophil From Bushmaster And Control Subjects Tested at Pre- and Post Exercise Periods

Number of Cells Adhering $\times 10^2$ Mean $\pm$ SEM(No. of Subjects)				
<u>Study Subjects</u>	<u>PRE-EXERCISE 1</u>	<u>PRE EXERCISE 2</u>	<u>POST EXERCISE 1</u>	<u>POST EXERCISE 2</u>
Bushmaster	11.78 $\pm$ 0.97 (19)	12.93 $\pm$ 1.21 (17)	14.30 $\pm$ 1.59 (15)	11.48 $\pm$ 1.0 (7)
Control	14.17 $\pm$ 1.34 (8)	19.10 $\pm$ 2.18 (8)	9.95 $\pm$ 1.51 (6)	ND
Percent Change	-16.9%	-32.3%	+30.4%	---

  

Oxidative Burst of Resting Cells Mean $\pm$ SEM ( No. of Subjects )				
	<u>PRE-EXERCISE 1</u>	<u>PRE EXERCISE 2</u>	<u>POST EXERCISE 1</u>	<u>POST EXERCISE 2</u>
Bushmaster	119.4 $\pm$ 13.5 (19)	167.4 $\pm$ 12.9 (18)	203.1 $\pm$ 14.1 (15)	171.76 $\pm$ 7.6 (4)
Control	131.3 $\pm$ 15.0 (8)	173.0 $\pm$ 25.8 (8)	176.3 $\pm$ 15.7 (6)	ND
Percent Change	-9.1%	-3.2%	+13.2%	---

  

Oxidative Burst FMLP ( $10^{-7}$ M ) Mean $\pm$ SEM ( No. of Subjects )				
	<u>PRE-EXERCISE 1</u>	<u>PRE EXERCISE 2</u>	<u>POST EXERCISE 1</u>	<u>POST EXERCISE 2</u>
Bushmaster	393.3 $\pm$ 32.3 (18)	465.9 $\pm$ 25.9 (18)	524.8 $\pm$ 20.3 (15)	514.9 $\pm$ 50.6 (4)
Control	325.6 $\pm$ 43.4 (9)	371.6 $\pm$ 49.3 (8)	366.7 $\pm$ 72.9 (6)	ND
Percent Change	+17.2%	+20.2%	+30.1	---

Of particular interest in these studies was the observation that oxidative burst reactions of Bushmaster neutrophils demonstrate a marked increase in reactivity and this increase reflects a time related event with exercise. This increase in oxidative burst activity occurred predominantly in Bushmaster neutrophils which had been stimulated with the chemoattractant, FMLP, suggesting that the neutrophils may have been "primed" or activated prior to collection. It has become apparent that neutrophils can exhibit different states of reactivity to stimulation. Emphasized first with bacterial endotoxin (lipopolysaccharide) (7) but now extended to a variety of stimuli, the cells can apparently be "primed" in a manner that does not cause the response in and of itself but does result in the subsequent response to another (or even sometimes the same) stimulus being of much greater magnitude. Such activation may occur through complement (C5) derived peptides, resulting in activation of NADPH oxidase prior to their collection, isolation and evaluation of these cells in the NBT assay.

Marked changes in oxidative metabolism ordinarily accompany perturbation in the plasma membrane or ingestion of particles by neutrophils. The cells consume increased amounts of oxygen to produce hydrogen peroxide, as well as several highly reactive, unstable intermediates such as superoxide anion radicals, hydroxyl radicals, and possibly, singlet oxygen. Concomitantly, there is stimulation of the hexose monophosphate shunt pathway of glucose oxidation and iodination of protein (mediated by the granule enzyme myeloperoxidase). The increased ability of neutrophils to reduce nitroblue tetrazolium dye is a reflection of enhanced generation of superoxide anion radicals.

There is considerable evidence that oxidative metabolism in neutrophils can be stimulated in the absence of phagocytosing. The first evidence that C5-derived peptides are capable of stimulating neutrophil oxidative metabolism was provided by Goetzl and Austen (8), who showed that purified human C5a increased, in a concentration-dependent fashion, the rate of aerobic glycolysis and hexose monophosphate shunt activity in human neutrophils. Subsequently, Goldstein et al. (9) demonstrated enhancement of nitroblue tetrazolium dye reduction by partially purified C5-derived peptides and enhancement of superoxide anion production by human neutrophils.

In addition bacterial endotoxin is a potent priming agent and its effect on neutrophils *in vivo* may lead to greater degrees of tissue injury. In this context, LPS

and chemotactic factors together have been shown to enhance neutrophil-dependent pulmonary vascular injury *in vivo* (10) and neutrophil-mediated damage to endothelial monolayers *in vitro* (11). The recent demonstration of a high correlation between the presence of both LPS and C5 fragments in the blood of patients at risk for adult respiratory distress syndrome with those who actually develop the syndrome (12) lends support to the suspicion that priming processes may contribute to inflammatory injury disease.

#### B. Rat Model of Cold Stress

A rat model of cold stress established to evaluate neutrophil functional response has resulted in the collection of data reflecting specific alterations in neutrophil activity suggestive of a cold related insult to the inflammatory system. Results of these studies are shown in Tables 4, 5 and 6.

**TABLE 4** Body Weight, Temperature and Hematologic Characteristics of Rats Exposed to 4°C and Matching Controls Held at 25°C.

Study Parameter	Temperature Exposure		P Value
	4°C	25°C	
	<u>Mean±SEM (No. of Animals)</u>		
Body Weight (grams)	241.5±3.5 (11)	238.1±3.5 (11)	NS
Body Temperature (Centigrade)			
Rectal	36.2±0.2 (14)	39.0±0.2 (12)	0.0001
Core	36.2±0.3 (14)	39.0±0.1 (12)	0.0001
White Blood Cell Number (X10 <sup>3</sup> /μl <sup>3</sup> )	5.1±0.3 (16)	3.3±0.2 (16)	0.0001
Neutrophil Percent (%)	9.9±1.6 (16)	12.8±1.3 (16)	NS
Neutrophil Number (X10 <sup>3</sup> /μl <sup>3</sup> )	0.50±0.09 (16)	0.44±0.07 (16)	NS

The effects of cold exposure were evident initially in body temperature measurement and changes in hematologic parameters (Table 4). Mean body temperature was 36.2°C for rats maintained at 4°C while rats held at 25°C had mean body temperatures of 39.0°C. White blood cell numbers were found to be higher in rats held at 4°C (mean 5.1 WBC/μl<sup>3</sup>) compared to numbers of rats held at 25°C (mean 3.3 WBC/μl<sup>3</sup>). Similarly, numbers of neutrophils from rats held at 4°C were higher (mean 0.50

neutrophils/ $\mu\text{l}^3$ ) than the number of neutrophils in rats maintained at 25 °C (mean 0.44 neutrophils/ $\mu\text{l}^3$ ).

Neutrophils isolated from rats held at 4°C adhered slightly more (mean 963.5 cells/well area) than neutrophils isolated from rats held at 25°C (mean 779.3 cells/well area) and this increase, although not significantly different, represented a 23.6% greater adhesive capability (Table 5). This may have importance since in postcapillary venules *in vivo*, there is some adherence of neutrophils to endothelial cells even in the absence of any inflammatory stimulus. In the absence of inflammation this association between neutrophils and endothelial cells *in vivo* appears to be reversible (13), influenced by fluid flow (14) and humoral factors such as epinephrine (15). The *in vivo* adherence of neutrophils to endothelium is greatly augmented by tissue damage and other inflammatory stimuli. In the presence of inflammation the adherent neutrophils subsequently migrate between endothelial cells into tissues and do not return to the circulation. With a more intense inflammatory process there is adhesion of circulating neutrophils to other neutrophils that have adhered to, but not migrated through, the endothelial cell layer. This process may result in occlusion of the blood vessel (16).

**TABLE 5.** Response Characteristics in Adherence, Migration and Oxidative Burst of Neutrophils from Rats exposed to 4°C and Matching Control Rats Exposed to 25°C

Study Parameter	Temperature Exposure		P Value
	4°C	25°C	
	Mean±SEM (No. of Animals)		
Adherence	963.5±145.7 (6)	779.3±114.2 (6)	NS
Oxidative Burst			
Resting (Response Score)	7.3±3.34(8)	8.3±1.3 (10)	NS
FMLP ( $10^{-7}$ M) (Response Score)	141.8±16.49(8)	238.5±22.3 (10)	0.001
PMA (0.1 nM) (Response Score)	141.8±20.9 (8)	244.0±32.21(8)	0.05
Migration			
Random (Number of Cells)	14.1±2.61(6)	44.2±13.4(5)	0.10
FMLP (Number of Cells)	51.3±8.7 (8)	92.9±21.2 (5)	0.05

The oxidative burst characteristics of neutrophils from rats held at 4°C were markedly lower (14.2% reduced) for resting cells i.e. cells not stimulated with a chemoattractant. Similarly, the oxidative burst reaction of cells stimulated with FMLP (32.1% reduced) or phorbol myelate acetate (PMA - 40.3% reduced) were significantly lower compared to responses of rats held at 25°C.

PMA activates the protein kinase C pathway leading to oxygen generation in the absence of membrane activation processes while FMLP stimulates oxidative burst reactions initially through membrane receptor mediated mechanisms (17). Reduced oxidative burst activity in both PMA and FMLP stimulated neutrophils may be related to suppressed oxygen generation pathways unrelated to the membrane receptor mediated events.

Early findings indicated that the respiratory burst of neutrophils was not needed for the generation of the metabolic energy required for phagocytosis; rather, the respiratory burst appeared to be required for optimum microbicidal activity as indicated by the decrease (but not loss) of microbicidal activity on exposure of neutrophils to hypoxic conditions (18) and by the association of a microbicidal defect (19) with the absence of the respiratory burst (20) in the leukocytes of patients with chronic granulomatous disease. This finding has focused attention on the enzyme systems responsible for the respiratory burst in particular NADPH oxidase (21) and on the nature of the toxic products formed.

The reduced capability to undergo optimum oxidative burst reactions was associated with a reduced capability of the cells from rats exposed to cold temperature to migrate both randomly (44.3% reduced ) and to FMLP (44.8% reduced ) compared to matched control rats maintained at 25°C (Table 5).

Previous authors have shown that the thyroid axis of rats respond rapidly to acute cold exposure and these responses include elevated serum levels of T<sub>3</sub> and falling levels of T<sub>4</sub> (22, 23 24). We have determined the serum level concentrations of thyroid axis hormones and corticosterone to document that the cold temperature induced expected changes in these parameters (Table 6). A significant increase in total T<sub>3</sub> concentration ( $p < 0.05$ ) was observed in treated rats compared to controls. In contrast, a 12% decrease ( $p > 0.14$ ) in T<sub>4</sub> concentration was observed in rats exposed to 4°C when compared to controls. There was a slight decrease in the concentration of corticosteron

following cold exposure compared to 25°C control rats. These findings are comparable with the reports of other cold model studies, for example, rats exposed to 4°C for four hours (24,25), and humans exposed to 4°C for two hours (22) exhibited elevated serum concentrations of T<sub>3</sub>, while concentrations of T<sub>4</sub> were reduced in both species. Although Baroni et al (26), have suggested a direct correlation between thyroxin and the immune system, correlation between thyroid activity and PMN function can not be established from these data.

**TABLE 6.** Thyroid Hormones, Corticosterone and Vitamin B-12 Binding Protein Concentrations in Sera of Rats Exposed to 4°C and Matching Control Rats Exposed to 25°C

Study Parameter	Temperature Exposure		P. Value
	4°C	25°C	
	Mean±SEM (No. of Animals)		
T <sub>3</sub> (ng/dl)	91.3±4.0	75.5±5.7	0.05
T <sub>4</sub> (ug/dl)	4.1±0.3(8)	5.0±0.5	0.14
TSH (ng/ml)	0.61±0.09	0.62±0.05	NS
Corticosterone (ng/ml)	364.1±6.8	933.2±4.9	NS
Vitamin B-12 Binding Protein Serum Level (pg/200 ul)	1855.8±66.5 (8)	1794.3±68.7 (8)	NS

Neutrophil specific granules are the most prevalent granules within the cell, and their contents are released into the circulation in response to an inflammatory stimulus (27). To determine whether cold exposure stimulated neutrophil degranulation, the sera of rats exposed to 4°C and those maintained at 25°C were evaluated for concentrations of B12-BP, one of the specific granule components of PMN. Data indicate that the concentration of B12-BP in the serum of treated rats ( 1855±66.5 pg B12-BP/ 200 µl serum ) was not altered by four hours of cold exposure as compared to levels found in the serum of control rats ( 1794±68.7 pg B12-BP/ 200 µl serum ) (Table 6). The concentration of B12-BP would not be expected to decrease during a four cold exposure period. However, neutrophil activation and subsequent degranulation and release of specific granules which contain the vitamin B12 binding protein may result from the cold insult (27). Recent data suggests that neutrophil

membrane fusion or degranulation is a step in the activation of oxidative burst (28), and in the presence of depressed oxidative burst activity in the neutrophils of cold exposed rats, an increase in degranulation may not occur.

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